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Murphy

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- [54] ACETATE ADDITION TO PLATELETS STORED IN PLASMA
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- [21] Appl. No.: **784,695**
- [22] Filed: **Oct. 30, 1991**
- [51] Int. Cl.⁵ **A01N 1/02**
- [52] U.S. Cl. **435/2; 424/533**
- [58] Field of Search **435/2; 424/533**

[56] **References Cited**

U.S. PATENT DOCUMENTS

Re. 32,874	2/1989	Rock et al.	424/101
4,447,415	5/1984	Rock et al.	424/101
4,695,460	9/1987	Holme	424/101
4,828,976	5/1989	Murphy	435/2
4,994,367	2/1991	Bode et al.	435/2

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 Cesar, Diminno, Alam, Silver and Murphy in "Plasma Free Fatty Acid Metabolism During Storage of Platelet Concentrates for Transfusion", *Transfusion*, 27(5): 434-437 1987.
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Simon, Nelson, Carmen and Murphy in "Extension of Platelet Concentrate Storage", *Transfusion*, 23: 207-212, 1983.

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[57] **ABSTRACT**

The present invention provides a composition and method for improving the storage of platelets and optimizing the viability of stored platelets. The present invention allows platelets to be stored in plasma for extended periods, without the addition of buffer, by adding acetate to a platelet concentrate.

4 Claims, 4 Drawing Sheets

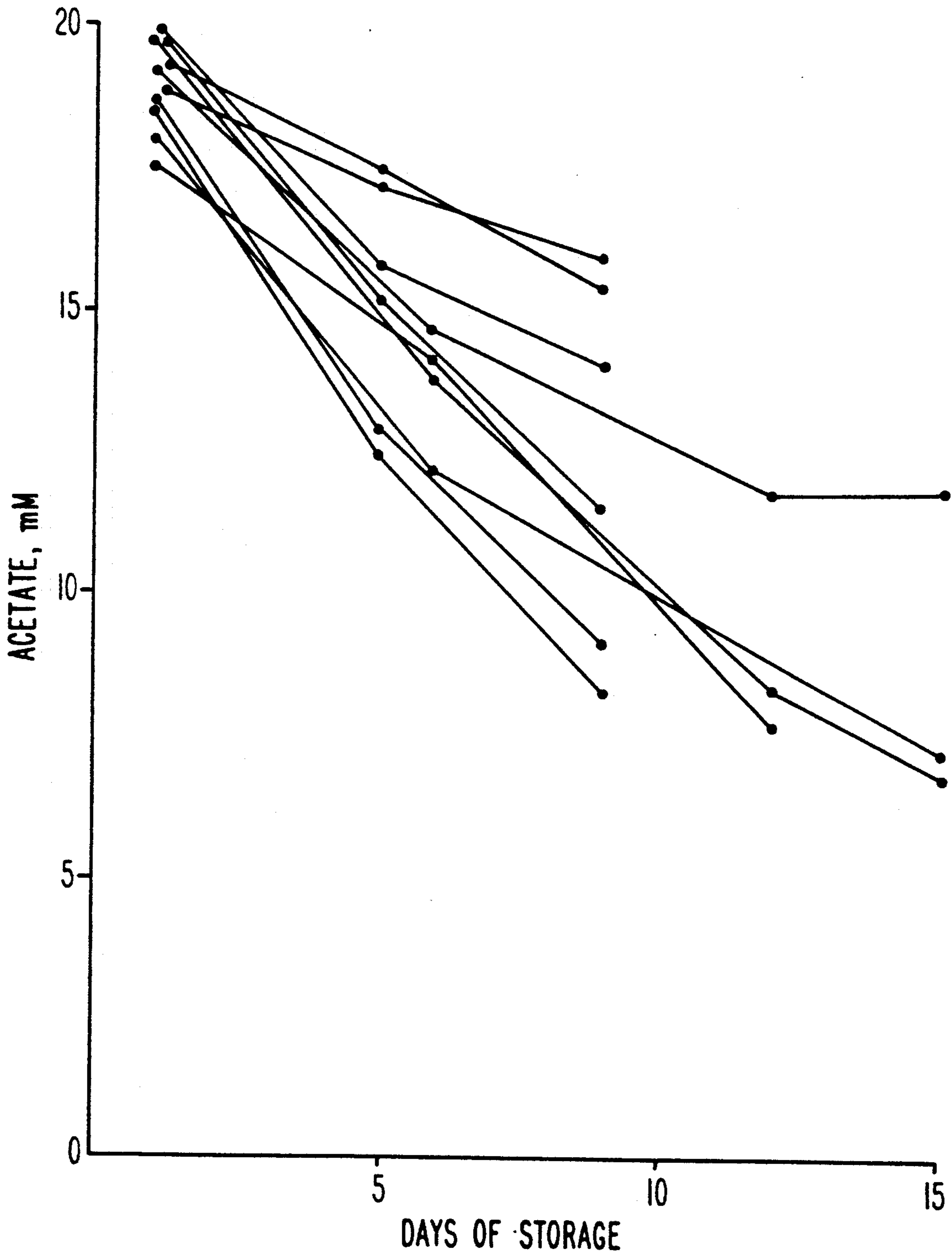


Fig. 1

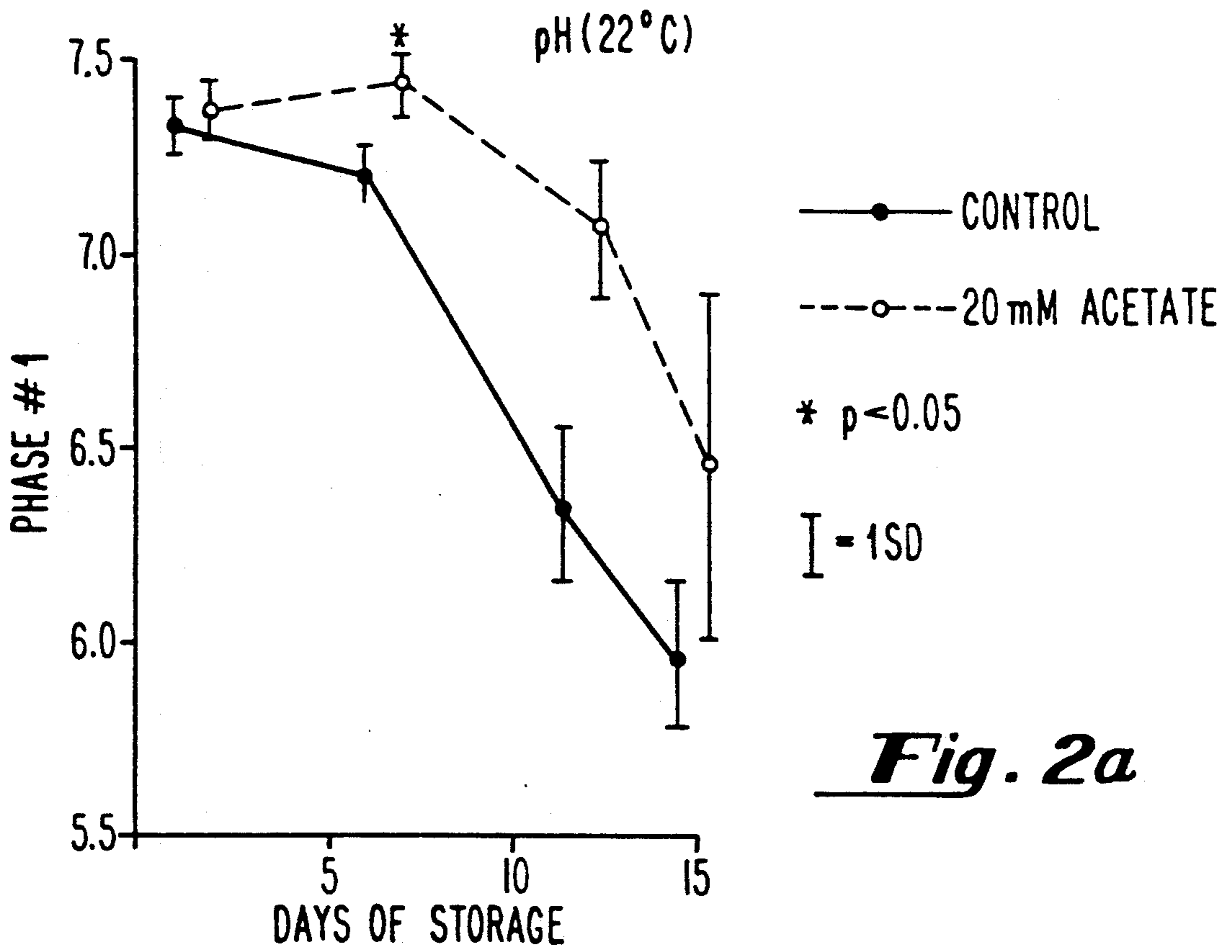


Fig. 2a

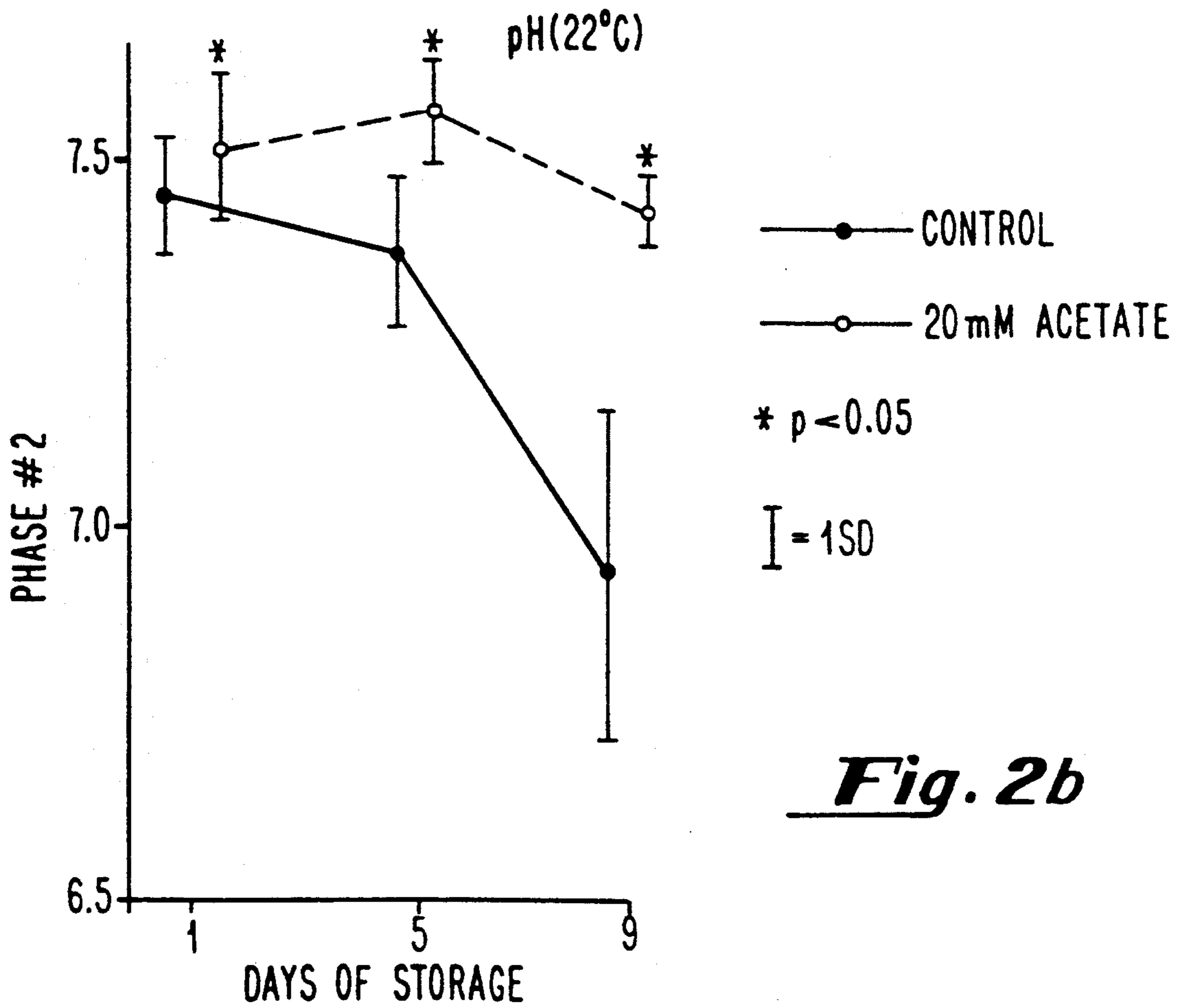


Fig. 2b

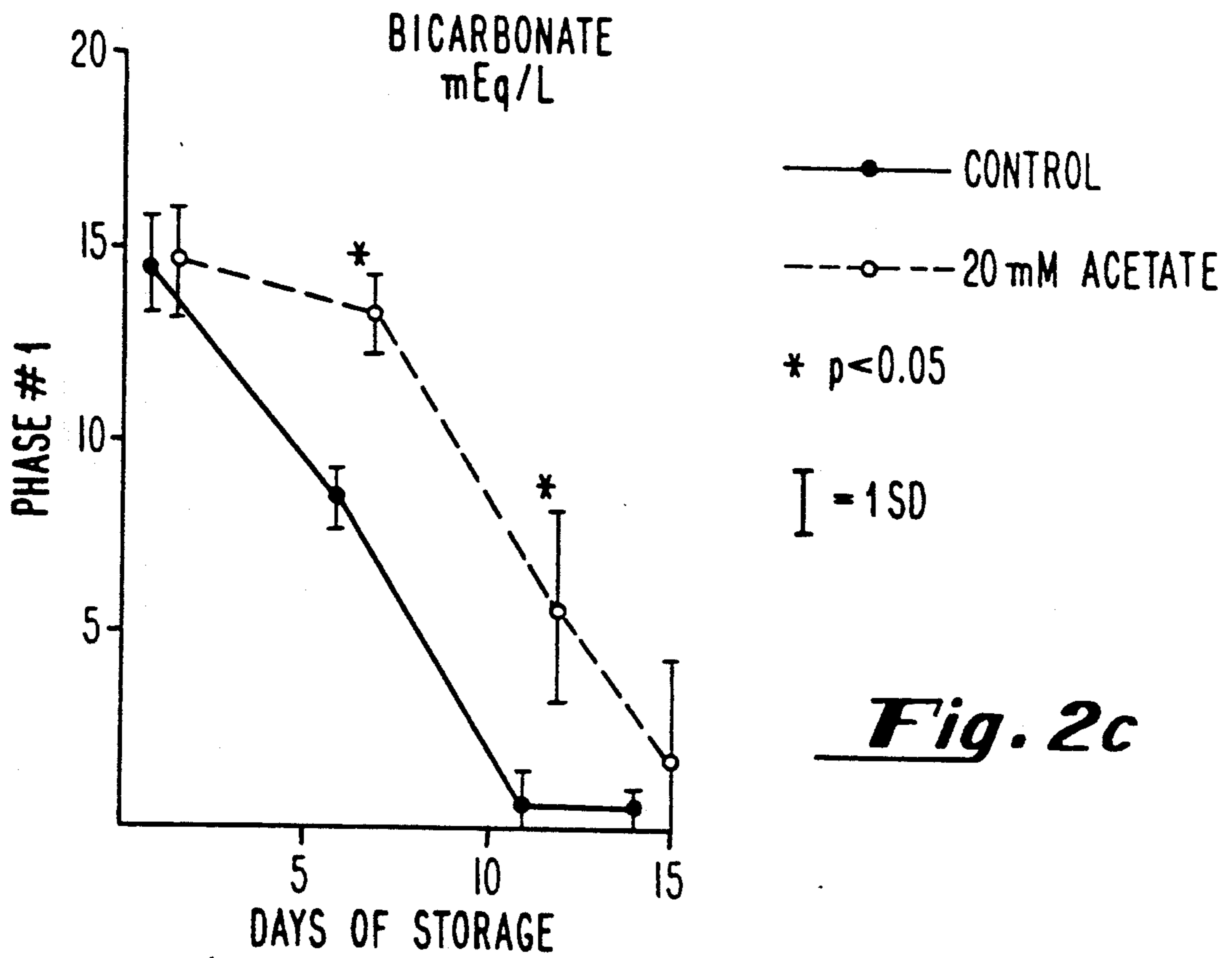


Fig. 2c

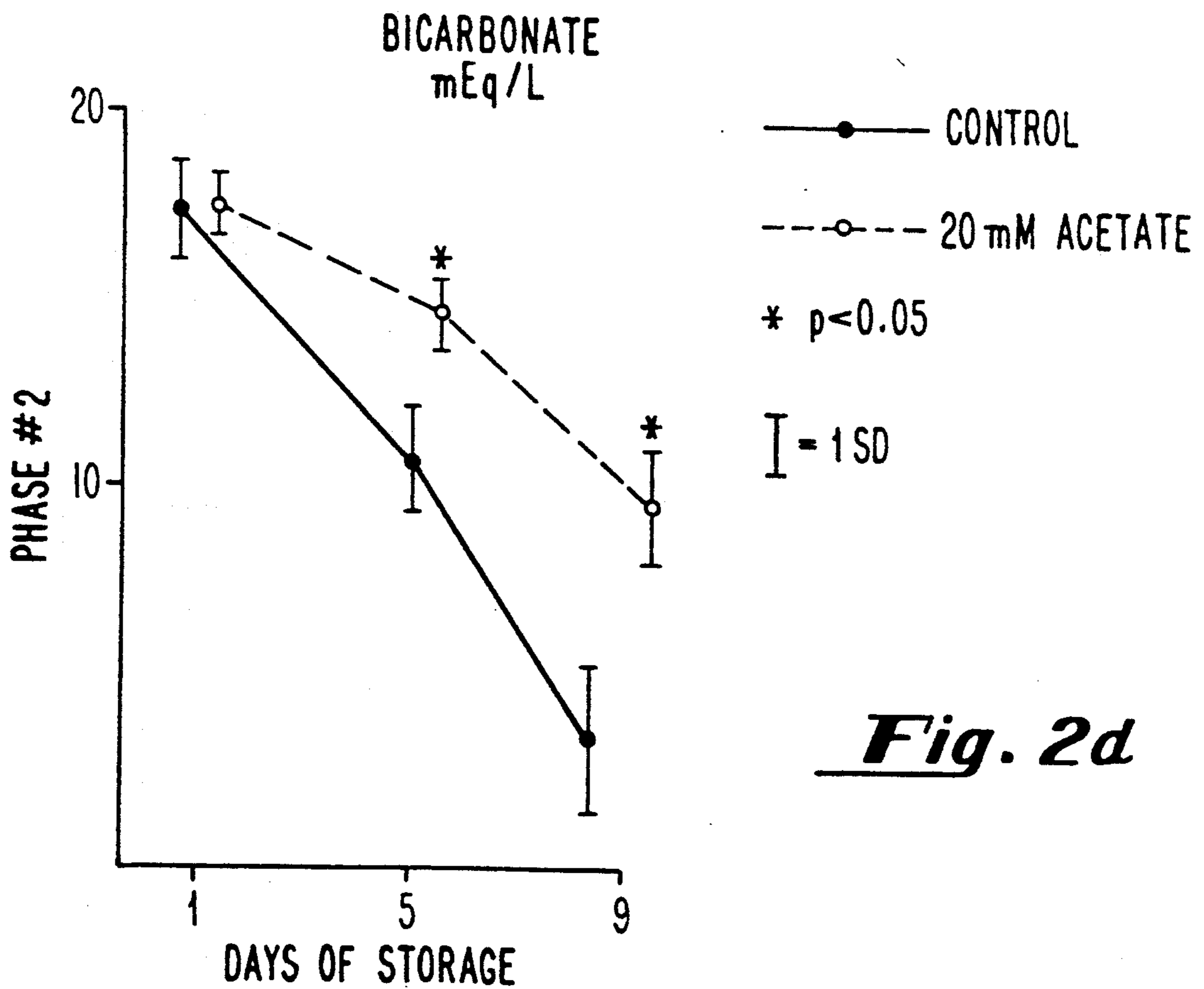


Fig. 2d

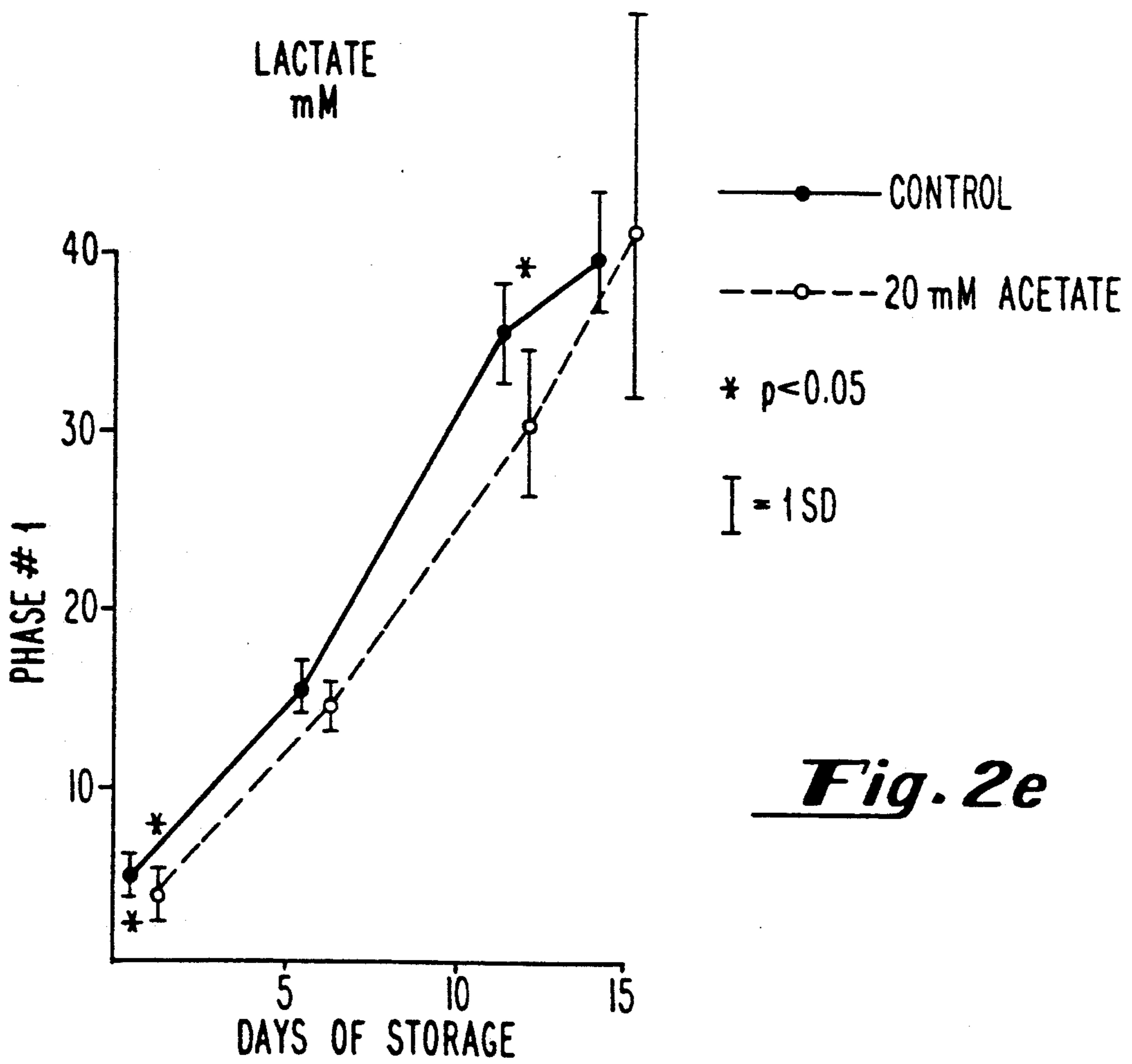


Fig. 2e

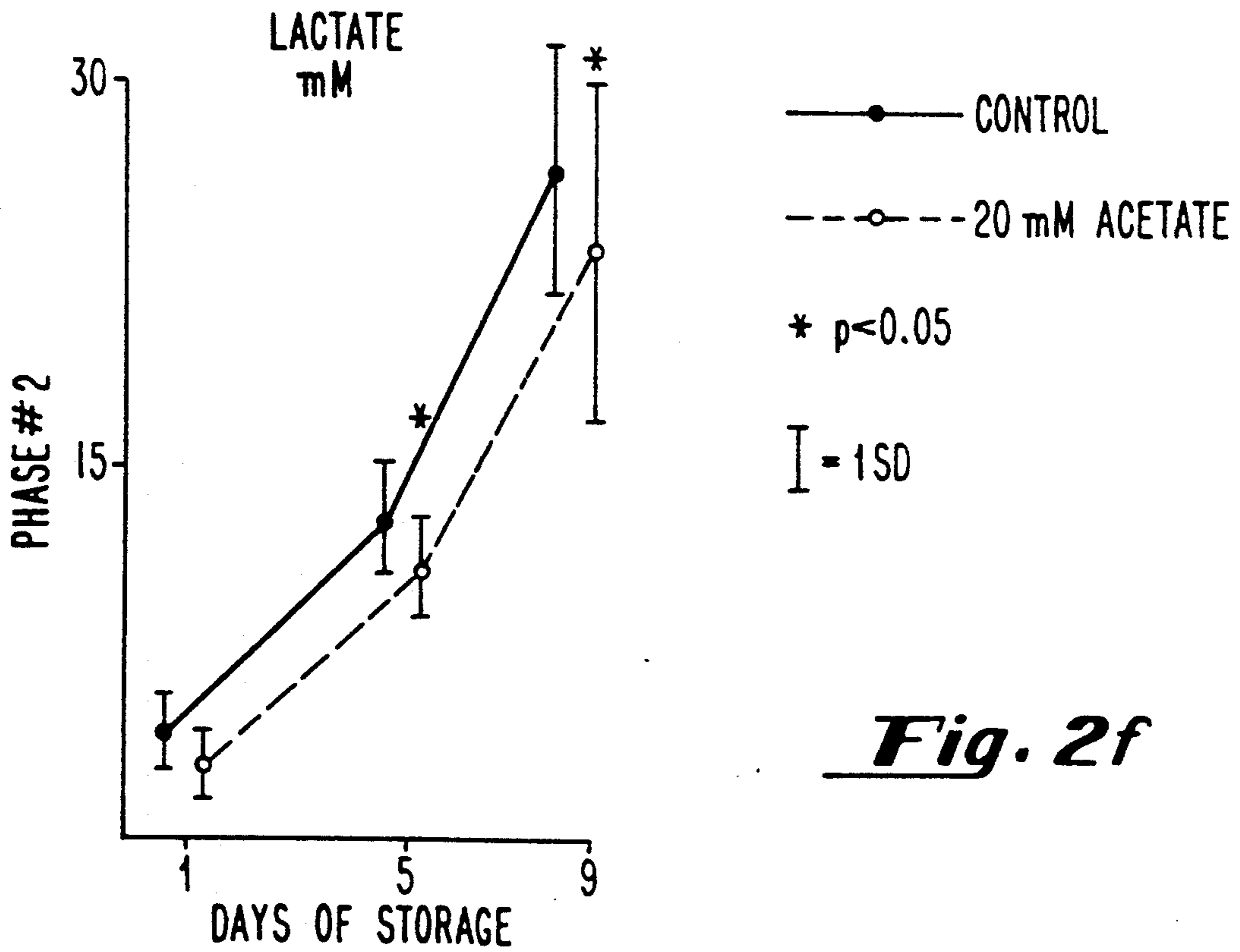


Fig. 2f

ACETATE ADDITION TO PLATELETS STORED IN PLASMA

GOVERNMENT SUPPORT

Portions of this work were supported by NIH Grant HL20818-15 from the National Institute of Health. The U.S. government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

The present invention relates to a composition and method to improve the storage of platelets prior to transfusion into a patient. Platelets are obtained as a by-product from whole blood donations and from plateletpheresis procedures. Typically, they are stored at $22 \pm 2^\circ$ C. in their own plasma within a plastic container whose walls are permeable to atmospheric gases. The plasma associated with these platelets normally contains all the ingredients of normal plasma plus ingredients in the primary anticoagulant which result in a dextrose concentration five times the physiologic concentration. The dextrose is added to the primary anticoagulant for the benefit of red cells which require it during storage, and dextrose is generally accepted to be required for platelet storage as well. In routine blood banking practice, the primary anticoagulant which is utilized is citrate-phosphate-dextrose (CPD), from Mollison, P. L., *Blood Transfusion in Clinical Medicine*, 7th Edition, Blackwell, 1983.

Donations of a unit of blood (63 ml of CPD mixed with 450 ml of whole blood) are processed by centrifugation into three fractions: red cells, plasma, and platelets. The volume of packed red cells from a unit of blood is approximately 180 ml, with a remaining volume of plasma and anticoagulant of about 333 ml. As used in the remainder of this application, the term plasma includes any anticoagulant which has been added thereto at the time of blood collection. The red cells are typically suspended in approximately 45 ml of plasma. Platelets are suspended in approximately 50 ml of plasma. This platelet containing product is typically referred to as a "platelet concentrate" (PC). The remaining 238 ml of plasma is frozen as fresh plasma.

A great deal is known about human platelet cells. General papers describing techniques, materials and methods for storage of platelets are described by Murphy et al. in "Improved Storage of Platelets for Transfusion in a New Container", *Blood*, 60(1):194-200 (July, 1982); by Murphy in "The Preparation and Storage of Platelets for Transfusion", Mammon, Barnhart, Lusher and Walsh, PJD Publications, Ltd., Westbury, N.Y. (1980); by Murphy in "Platelet Transfusion", *Progress in Hemostasis and Thrombosis*, Vol. III, Edited by Theodore Spaet, Grune and Stratton, Inc. (1976); by Murphy et al. in "Platelet Storage at 22° C.: Role of Gas Transport Across Plastic Containers in Maintenance of Viability", *Blood*, 46(2):209-218 (1975); by Kilkson, Holme and Murphy in "Platelet Metabolism During Storage of Platelet Concentrates at 22° C.", *Blood*, 64 (2):406-414 (1984); by Murphy in "Platelet Storage for Transfusion", *Seminars in Hematology*, 22(3):165-177 (1985); by Simon, Nelson, Carmen and Murphy in "Extension of Platelet Concentrate Storage", *Transfusion*, 23:207-212 (1983); by Cesar, Diminno, Alam, Silver and Murphy in "Plasma Free Fatty Acid Metabolism During Storage of Platelet Concentrates for Transfusion", *Transfusion*, 27(5):434-437 (1987); each of which publications is

hereby incorporated by reference and is more fully set forth herein.

There exists a considerable body of prior art concerning storage of platelets. Prior work has shown that the duration of platelet storage is limited by the continuing production of lactic acid from dextrose by the platelets. Although this provides energy for the platelets, the lactic acid acidifies the medium, which acidity eventually destroys the platelets. It has also been shown that platelets consume oxygen during storage for energy production, the end product of which process is a gas, CO_2 , which unlike lactic acid, can leave the platelet concentrate through the plastic walls of the container in which it is normally stored. The production of CO_2 does not acidify the storage medium for the platelets. In addition to the glycolysis of dextrose, fatty acids and amino acids typically present in the plasma may be used as substrates for oxidative metabolism of stored platelet cells.

Most platelet storage media contain glucose. In U.S. Pat. No. 4,695,460 (Holme), a synthetic platelet storage media is disclosed containing 3.0-7.5 grams of dextrose, 3.0-6.0 grams of sodium citrate, and 2.0-4.2 grams of sodium bicarbonate. U.S. Pat. No. 4,447,415 (Rock) discloses a number of platelet storage solutions. It has been appreciated that platelet storage in a medium essentially free of glucose could be advantageous. For example, in U.S. Pat. No. 4,828,976, Murphy discloses a glucose free media for storing blood platelets. To store platelets for periods in excess of 5 days, it is taught that the storage media should be essentially free of glucose. It is also disclosed that it is the presence of glucose that leads to the generation of lactic acid which adversely affects platelet viability.

The rapid loss of platelet function during storage presents a significant problem in blood banking. One approach for diminishing or delaying the loss of platelet function during storage has been the development of plasma-free storage media. For example, U.S. Pat. No. 4,695,460 (Holme) and U.S. Pat. No. 4,447,414 (Rock et al.).

Another approach has focused on the biochemistry of platelet activation and means to regulate platelet activation, which results in platelet lysis and death. U.S. Pat. No. 4,994,367 (Bode et al.) discloses a blood platelet preparation comprising blood platelets, an adenylate cyclase stimulator (Prostaglandin E1), a phosphodiesterase inhibitor (Theophylline), a thrombin inhibitor (N-(2-naphthylsulfonyl)glycyl)-D,L-amidinophenylalaninipiperidide), and a plasmin inhibitor (Aprotinin). A plasma-free platelet storage medium comprising dextrose, sodium citrate, sodium bicarbonate, and a platelet activation inhibitor (adenylate cyclase stimulator) is also disclosed. A process for preparing a plasma-free platelet preparation by producing platelet-rich plasma (PRP) from whole blood, adding a platelet activation inhibitor (adenylate cyclase stimulator), centrifuging the PRP to deposit the platelets on the bottom of the centrifuge container, removing the platelet-free plasma supernatant, and adding a plasma-free liquid platelet storage medium is also provided.

The adenylate cyclase stimulator is included to increase the production of adenosine 3', 5'-cyclic phosphate (cAMP) in the blood platelets. The phosphodiesterase inhibitor is included to reduce the degradation of cAMP in the blood platelets. The thrombin inhibitor is included to reduce the stimulation of the blood plate-

lets. The plasmin inhibitor is included to reduce the degradation of cell surface proteins on the blood platelets.

Heaton et al., *British Journal of Hematology*, 75:400-407 (1990) disclose an ionically balanced electrolyte solution with citrate, glucose and bicarbonate which was shown to provide good platelet viability with storage for up to 7 days. When adenine was added to this solution, it also allowed for satisfactory preservation of red cells for extended periods.

A major problem of platelet storage in synthetic media is the potential for pH fall resulting from the lactate end product of glycolytic energy metabolism. The approach taken here is to add bicarbonate to buffer the acid load generated by a glucose-containing medium. In vivo studies demonstrated improved post-transfusion viability with platelets stored in this medium as compared to CPD-plasma.

Guppy et al., *Vox Sanguinis*, 59:146-152 (1990) studied the metabolism of platelets in vitro. It was found that glucose is never oxidized to any significant extent and is always converted to lactate, regardless of oxygen availability. Preliminary storage experiments using plasma-free media showed that an acetate-containing buffered salt solution provided excellent storage conditions and that a medium without any exogenous fuel is better than one containing glucose. Thus, it is concluded that a platelet storage medium should contain minimal amounts of glucose, and an oxidizable fuel in order to supplement the endogenous one. The identity of this fuel is not known; however, it is shown not to be glucose or glycogen. It is concluded that platelets can use acetate and when present, this fuel completely replaces the endogenous fuel. The data suggest that acetate, short chain fatty acids or amino acids metabolizable through the TCA cycle should provide ATP efficiently at low molarities without producing toxic end-products.

Notwithstanding the considerable work conducted in this area, a need still exists for means to improve the storage of platelets in a viable condition.

SUMMARY OF THE INVENTION

The present invention provides a composition and method to improve the storage of platelets in plasma. In plasma, a limiting factor for long term storage is that lactic acid production exceeds the bicarbonate buffering capacity leading to lethal pH fall at day 10-14 of storage. The present invention allows platelets to be stored in plasma for a longer period of time prior to the occurrence of pH fall, due to lactic acid production. Therefore, this invention would be useful to prolong the interval during which viable platelets can be stored in plasma.

Accordingly, the present invention provides for the addition of acetate to a platelet concentrate. Addition of acetate results in acetate being oxidized by the platelets resulting in bicarbonate formation. Thus, storage can be extended due to the better preservation of pH. As a result, the invention improves platelet transfusion therapy.

A further object of the present invention is the use of acetate to optimize platelet storage.

This and further objects of the present invention will become apparent from the following more detailed description.

DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing acetate concentration (in mM) versus the days of storage.

FIG. 2(a-f) shows the results of studies with pairs of PC showing changes in pH, bicarbonate, and lactate with days of storage.

DETAILED DESCRIPTION OF THE INVENTION

In order to maintain viability, platelets must continuously generate new adenosine triphosphate (ATP) to meet their energy needs. Two pathways are normally available: glycolysis and oxidative phosphorylation. In glycolysis, one molecule of glucose is converted to two molecules of lactic acid to generate two molecules of ATP. In oxidation, glucose, fatty acid or amino acid enters the citric acid cycle and is converted to CO₂ and water. This pathway requires the presence of an adequate supply of oxygen. It is much more efficient than glycolysis. Oxidative metabolism of glucose to CO₂ and water yields 36 molecules of ATP.

It has been recognized that platelets will meet their energy needs in a manner which is not necessarily consistent with their long term storage in a viable condition. When given adequate oxygen, platelets produce most of their ATP through oxidation, but continue to produce lactic acid instead of diverting all metabolized glucose through the oxidative pathway. During the storage of platelets in plasma, lactic acid concentrations rise at approximately 2.5 mM per day. See Murphy et al.; "Platelet Storage at 22° C.", *Blood*, 46(2):209-218 (1975); Murphy, "Platelet Storage for Transfusion", *Seminars in Hematology*, 22(3):165-177 (1985). This leads to gradual fall in pH. As explained in the aforementioned Murphy articles, when lactic acid reaches about 20 mM, the pH which started at 7.2 may reach 6.0. Since platelet viability is irreversibly lost if pH falls to 6.1 or below, a major limiting variable for platelet storage is pH. At this rate of lactic acid production, pH would fall much more rapidly if it were not for naturally occurring plasma buffers, principally sodium bicarbonate.

In the present invention, acetate is added to optimize platelet viability during storage. To examine the effect of addition of acetate on platelets during storage in plasma, a donor underwent double plateletpheresis to obtain paired PC. For each paired study, sodium acetate was added to one of each pair in an amount calculated to achieve a concentration of 20 mM. The range of measured concentrations on day 1 of storage was 17-20 mM (n=10). The stored platelets were assessed by standard techniques such as platelet count, platelet volume, dispersion, extent of shape change, platelet aggregation, and microscopic morphology. The present invention therefore provides a composition and method which optimizes platelet viability.

The invention is illustrated in the following, non-limiting example.

EXAMPLE 1

In ten studies, a pair of platelet concentrates (PC) were prepared from a volunteer, normal donor using techniques previously described by Murphy (1980). 0.6 ml sodium acetate (2Eq/L, Abbott Labs, Chicago) was added to one of each pair. This amount was calculated to achieve a 20 mM concentration in the PC at the initiation of storage. Measured acetate concentrations

were in the range of 17–20 mM, after 24 hours of storage. FIG. 1 shows that after 24 hours, the concentration of acetate fell continuously from day 1 to at least day 12 of storage. The higher rates of fall were seen in those PC with the higher concentration of platelets.

The study had two phases. In Phase 1 (4 pairs of PC), storage was carried out for 15 days with measurements made on days 1, 6, 12, and 15. In Phase 2 (6 pairs of PC), storage was carried out for 9 days with measurements made on days 1, 5, and 9. The results are shown in FIG. 2. From day 5 through day 12 of storage, pH and bicarbonate concentration were significantly higher in the PC which received acetate. The falls in pH and bicarbonate concentration were delayed by 4–5 days in the PC which received acetate, relative to the PC which did not. Thus, the addition of acetate is capable of prolonging the duration of storage by 4–5 days. FIG. 2 also shows that acetate slowed the production of lactic acid.

Detailed data from the two phases of the study are provided in Tables 1 and 2.

The rate of oxygen consumption (O_2CON) is greater in the PC with acetate, indicating that a fuel for oxidative metabolism has been added. The other measurements, ATP, osmotic reversal reaction (Os Rev), % discs by phase microscopy, platelet count and mean platelet volume (MPV) as percentage of day 1 value, and dispersion (DISP) of the platelet size distribution, all reflect platelet quality. On day 12 (Phase 1), ATP, % discs, and maintenance of MPV are all superior in the PC which received acetate, reflecting the superior maintenance of pH when acetate is added to the platelet preparation.

What is claimed is:

1. A method of storing a human blood platelet preparation consisting essentially of: providing a human blood, plasma-based platelet concentrate comprising platelets suspended in a solution consisting essentially of plasma, the pH of the preparation being at least 6.1; and

TABLE 1

	PHASE 1							
	Day 1		Day 6		Day 12		Day 15	
	A	no A	A	no A	A	no A	A	no A
pH	7.37 ± 0.04	7.31 ± 0.03*	7.44 ± 0.04	7.21 ± 0.07*	7.08 ± 0.18	6.33 ± 0.20*	6.44 ± 0.47	5.95 ± 0.17
HCO ₃	14.6 ± 1.5	14.5 ± 1.0	13.3 ± 0.7	8.5 ± 0.4*	5.5 ± 2.3	1.0 ± 0.6*	1.8 ± 2.6	0.3 ± 0.1
LACTATE	4.1 ± 0.5	5.0 ± 0.7*	14.3 ± 1.8	15.6 ± 1.1	30.1 ± 4.5	35.6 ± 2.6*	41.2 ± 8.5	39.9 ± 3.6
O ₂ CON	1.25 ± 0.15	0.97 ± 0.25*	0.89 ± 0.28	0.85 ± 0.23	0.60 ± 0.26	0.26 ± 0.23	0.15 ± 0.30	0.08 ± 0.17
O ₂ CON-%1	—	—	70.7 ± 17.7	83.9 ± 12.4	47.3 ± 18.8	25.8 ± 19.3	12.5 ± 25.1	7.5 ± 14.9
ATP	2.91 ± 0.19	2.93 ± 0.54	2.25 ± 0.15	2.31 ± 0.66	1.39 ± 0.53	0.82 ± 0.66*	0.55 ± 0.56	0.14 ± 0.04
ATP-%1	—	—	77.1 ± 0.7	77.6 ± 9.4	47.5 ± 17.2	26.6 ± 18.4*	18.7 ± 19.3	4.6 ± 0.8
Os Rev	64.6 ± 11.2	62.0 ± 15.1	46.9 ± 7.1	48.2 ± 9.7	27.0 ± 17.4	14.9 ± 19.1	13.3 ± 14.8	2.1 ± 2.4
Os Rev %1	—	—	72.9 ± 3.6	78.5 ± 5.5	39.5 ± 19.9	20.9 ± 25.9	18.5 ± 18.9	3.2 ± 3.6
% Discs	82.5 ± 9.6	85.0 ± 10.0	62.5 ± 35.9	60.0 ± 38.3	32.5 ± 39.8	10.0 ± 14.1*	16.3 ± 29.3	0.0 ± 0.0
PLCT %1	—	—	95.1 ± 3.9	87.8 ± 8.8	83.2 ± 6.2	78.1 ± 13.6	74.1 ± 4.6	76.3 ± 13.3
DISP	1.72 ± 0.11	1.79 ± 0.18	1.79 ± 0.07	1.82 ± 0.15	1.91 ± 0.14	1.98 ± 0.19	2.16 ± 0.25	2.24 ± 0.22
MPV %1	—	—	98 ± 4	104 ± 6*	104 ± 4	139 ± 13*	152 ± 43	164 ± 20

data presented as means ± 1 SD

*p < 0.05, paired t-test

N = 4

TABLE 2

	PHASE 2					
	Day 1		Day 5		Day 9	
	A	no A	A	no A	A	no A
pH (22)	7.51 ± 0.10	7.45 ± 0.08*	7.56 ± 0.10	7.38 ± 0.10	7.43 ± 0.06	6.94 ± .21*
HCO ₃	17.9 ± 0.84	17.8 ± 1.3	14.6 ± 1.0	10.7 ± 1.3*	9.5 ± 1.6	3.4 ± 1.4*
LACTATE	3.6 ± 1.2	4.3 ± 0.9	11.3 ± 2.2	13.1 ± 2.1*	22.0 ± 5.9	25.0 ± 4.6*
O ₂ CON	1.55 ± 0.22	1.36 ± 0.23	1.33 ± 0.19	1.04 ± 0.15	1.04 ± 0.21	0.92 ± 0.22
O ₂ CON-%1	—	—	85.9 ± 8.0	77.3 ± 3.7*	66.5 ± 7.6	67.8 ± 13.22
ATP	3.32 ± 0.48	4.12 ± 0.56	3.26 ± 0.70	3.15 ± 0.26	2.47 ± 0.79	2.32 ± 0.51
ATP-%1	—	—	98.1 ± 11.1	77.7 ± 13.1	74.9 ± 23.8	56.7 ± 11.7
Os Rev	66.8 ± 13.4	66.1 ± 9.8	46.6 ± 2.9	53.5 ± 7.9*	39.0 ± 4.4	40.6 ± 8.3
Os Rev %1	—	—	71.5 ± 11.3	81.4 ± 10.7	59.9 ± 11.3	61.4 ± 9.1
% Discs	88.3 ± 2.6	80.0 ± 16.7	60.8 ± 24.6	66.7 ± 21.6	43.3 ± 31.4	39.2 ± 28.7
PLCT %1	—	—	96.2 ± 2.1	94.1 ± 2.4*	87.4 ± 3.6	88.3 ± 6.8
DISP	1.67 ± .01	1.67 ± .03	1.68 ± .03	1.66 ± .02*	1.71 ± .03	1.68 ± .01
MPV %1	—	—	104 ± 5	103 ± 4	107 ± 5	115 ± 11

data presented as means ± 1 SD

*p < 0.05, paired t-test

N = 6

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adding acetate to the platelet concentrate in an amount sufficient to reduce the pH fall of the preparation and to maintain the pH of the preparation above 6.1.

2. The method of claim 1 wherein the step of adding the acetate is performed so as to ensure that the amount of acetate in the platelet preparation is about 20 mM.

3. The method of claim 1 further comprising main-

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taining the pH of the platelet preparation above 6.8 for a period of 12 days.

4. The method of claim 1 wherein the step of adding the acetate is performed so as to ensure that the amount of acetate in the platelet preparations is from about 17-20 mM.

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